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Formulation and evaluation of novel gelling agent as a probiotic delivery system

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A Dissertation

submitted in partial fulfilment

of the requirements for the Degree of

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by

Jithin Kandan Chirakkal

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Master of Science in Food innovation.

Formulation and evaluation of novel gelling agent as a probiotic delivery system

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Jithin Kandan Chirakkal

Probiotics are gaining importance day by day and are being added into the diet with various mediums such as yogurt, butter and other diary products. However, the shelf life of the organism and all not much preserved in those scenarios if the packaging and the logistics are not good enough. So, the food technologists were finding ways to tackle this issue by trying to incorporate probiotic microbes into edible gels. Many researcher's have succesfully engineered hydrogels which are capable enough to carry the probiotic organisms down the gastrointestinal tract. Most of the hydrogels used for delivery of probiotics use gelatine as plasticisers, but it is being manufactured from bovine or porcine sources, which is unaccepatable for certain religious and cultural groups. Thus, syntheisis a novel gel (NG) from completely from non animal sourced food grade material to become a probiotic delivery agent both topically and systemically. NG has been syntheisied by combining citric acid (CA) and disodium 5-guanylate (DG) in water forming a viscoelastic gel with good textural property to render itself as a profound topical probiotic agent for acne and other skin disorders. During the rheological analysis, NG was showing similar thixotropic behaviour as gelatine. Viscometric analysis of NG was carried out at different concentrations and at different tempertaure. NG was showing higher viscosity when stored at 4°C. Also, when the concnetration of CA and DG was varied there was a significant change in the rheology and textural property. This might be due to the hydrogen bond formation kinetics taking place when there is change in tempertaure. Benzac AC® anti acne gel was anylsed alongside with the NG and found that it has better cohessivness than NG but lacked hardness. However, the learning was to further research and formulate NG with a food grade polymer such as hydroxypropyl methylcellulose (HPMC) to get good adhessive property so as to act as topical probiotic delivery agent.

Keywords: Novel gelling agent, gelatine alternative, probiotic delivery

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"GOD the great giver opens the whole universe to our gaze in the narrow space"

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Chapter 1

Introduction

The skin is regarded as the largest organ in human body and is a residing place for intriguing microbiota, which withstand the harsh environment the skin offers (Rosenthal, Goldberg, Aiello, Larson, & Foxman, 2011). The high diversity of the bacterial community present in the skin has been revealed by metagenomics using 16S rRNA sequencing. The microbial community of the skin have interpersonal variation depending on the age, individual, body site and time. The four most dominant phyla are Firmicutes, Actinobacteria, Bacteroidetes and Proteobacteria. Out of these, 60% of the microbial population is accounted by species like, Propionibacterium, Staphylococcus and Corynebacterium (Scharschmidt & Fischbach, 2013).

As a first line of defence these resident microbes interact with the external microbes, such as pathogens. These bacteria through their molecular mechanisms produce antimicrobial agents which promote the inherent barrier function of the skin by boosting the activity of T cells and Langerhans cells (Y. E. Chen & Tsao, 2013). Dynamic and complex nature of the skin ecosystem is decided by an array of biochemical and physical aspects which can thus easily be disturbed. Therefore, any change in this bacterial equilibrium due to the alteration of the composition of skin bacteria, or a host immune response change can induce extensive inflammation of the skin (Scharschmidt & Fischbach, 2013). This dysbiosis leads to the advancement of pathologic skin conditions like acne, eczema, rashes, psoriasis, and dermatitis.

Among new agents which are extensively used and studied for health promoting effects, probiotics re the widely used ones out of all. Probiotics can be a single strain of organism or a mixture of different strains which can boost the immune system, enhance anti-inflammatory action, improve wound healing by helping in accumulating macrophages and lymphocytes at the wound sites (Oelschlaeger, 2010). Therefore, an innovative approach with probiotics can improve healing and fairly eliminate pathogenic microbes.

Probiotics is defined as, “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host” by Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO, 2006). In addition to the long know gastrointestinal health benefits of probiotics, they also help reducing skin related issues like eczema (Rautava, Kalliomäki, & Isolauri, 2002), reduce cholesterol (Wang, 2009) and also improves immunity (Tuohy, Probert, Smejkal, & Gibson, 2003). The benefits of probiotics do not stop here, it extends up in reducing colon and breast cancer risks too (Aragón, Perdigón, & de Moreno de LeBlanc, 2014; Liong, 2008). Various reasons like unhealthy eating habits, antibiotics and stress can cause reduction in probiotic levels, therefore

probiotics should be taken along with the diet (Govender et al., 2014; Varankovich, Nickerson, & Korber, 2015). The biggest issue faced by the formulators is the viability of the probiotic bacteria during the food processing and storage (McClements, 2017). Likewise, to complete their purpose, probiotics should survive the acidic condition the stomach and has to release in very high amount (Tripathi & Giri, 2014). Thus, a stable delivery system is needed to enhance the viability of probiotics in the gastrointestinal tract and during storage (Corona-Hernandez et al., 2013). As a probiotic delivery system many natural and synthetic polymers are used such as gelatin, polyvinyl alcohol or Eudragit (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012; de Barros, Scherer, Charalampopoulos, Khutoryanskiy, & Edwards, 2014; J. Kim, Muhammad, Jhun, & Yoo, 2016). Though, polysaccharide-based hydrogels are most used for encapsulation of probiotics.

In this work the focus was to evaluate the physical characteristic of the NG along with comparing it with a marketed anti acne gel to get insights about the topical stability. When considering a topical therapy most important thing to consider would be the formulation of a delivery system that could provide a close contact with the biological tissue and the active ingredient (Akomeah, 2010). A model topical delivery system will posse's supreme functionality amalgamated with good aesthetic properties. It should have good adhesive property, elasticity, concealed appearance, and durability along with complete protection from external environment including other microbes (Alsarra, 2009). The topical delivery destined for the application on to the skin should possess good mechanical property like spreadability, ability to attach to the skin for a long time (bio adhesion), good textural and rheological property. Also, most importantly the ability to release the active ingredient to the site of application. Flexing of the skin around the site of application is the biggest challenge faced by the delivery system which will directly affect the therapeutic effectiveness (Alsarra, 2009; Jones, Woolfson, & Brown, 1998). Hydrophilic polymers are gaining popularity as good wound healing agents, especially for local applications like acne and rashes. Additionally, hydrogels are easily washable unlike the sticky ointments after the desired therapeutic effect has been achieved (Boateng, Matthews, Stevens, & Eccleston, 2008). Moreover, high molecular weight, easy oxygen permeability, excellent moisturising potential along with mechanical properties mimics the soft tissue like skin. All these traits are very imperative for healing and due to the hydrophilic nature of such polymers actual trap a lot of moisture which in turn help in improved wound healing (Ribeiro et al., 2009; Sezer et al., 2008).

Chapter 2

Review of literature

2.1 Probiotics in systemic and surface immunity

2.1.1 Surface immunity

Immunity provided by probiotics for protecting ulcers or inflammation from pathogens is in a diversified manner (Figure 1). Probiotics and pathogens compete for the binding site at the adhesion sites on the host cells. This kind of binding encourage the host cells to produce anti-inflammatory chemicals like cytokines recuing the surface inflammation. Also, probiotics can help release many antimicrobial agents which can inhibit or eliminate many pathogens. Moreover, probiotic microbes can release wide range of antimicrobial agents which can inhibit or eliminate multiplication of pathogens. For instance, lactic acid produced by lactobacillus species alters the pH which in turn affects the growth of pH sensitive microbes. Binding to the toxins produced by the pathogens and rendering it inactive is another mechanism by which probiotics help in surface immunity. Comparable protection can be seen between microbiota in a symbiotic manner at different body parts such as skin, gut, oral cavity, and urogenital tract (Singh, Ahmad, Musarrat, Ehtesham, & Hasnain, 2013).

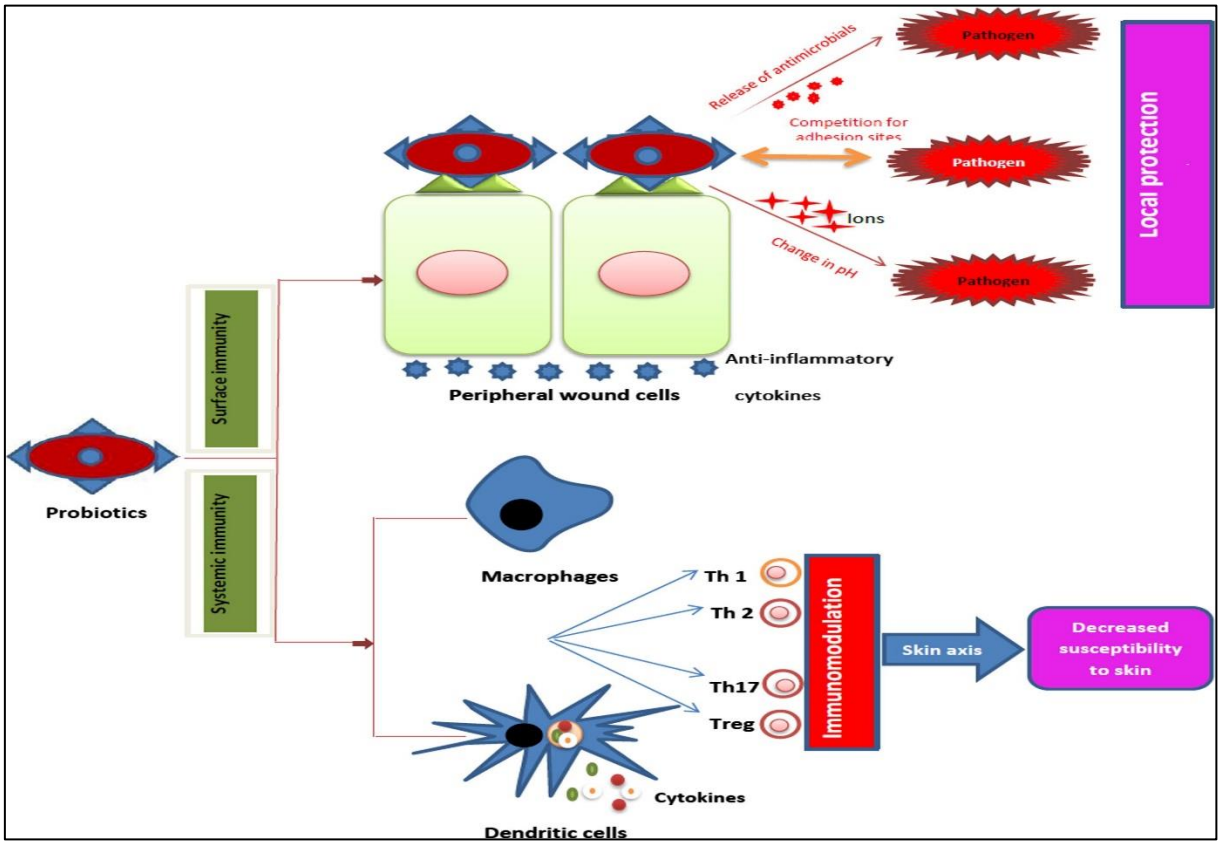

Figure 1: Immunomodulating mechanism of probiotics
(Sonal Sekhar, Unnikrishnan, Vijayanarayana, Rodrigues, & Mukhopadhyay, 2014)

2.1.2 Systemic immunity

Through immunomodulation probiotics can improve general body immunity (Figure 1). To achieve this, probiotic microbes interact with macrophages and dendritic cells (antigen presenting cells), releasing some mediators like cytokines regulating the function of T cells inducing immunomodulation systemically (Singh et al., 2013).

2.2 Hydrogels and hydrocolloids as probiotic carriers

The property of adhesion is very imperative when it comes to wet and dynamic surfaces such as biological tissues (J. Li et al., 2017). So, adhesives which can bind to these tissues effectively possess a wide array of potential in tissue repair (Duflo, Thibeault, Li, Shu, & Prestwich, 2006; Sharma et al., 2013) and drug delivery (J. Li & Mooney, 2016; Prausnitz & Langer, 2008). Hydrogel possess a vast crosslinking and good swelling index, along with hydrophilic polymer structure minimises the biocompatibility issues effectively (Freedman & Mooney, 2019; Kamata, Li, Chung, & Sakai, 2015). In many therapeutic applications the hydrogels are extensively studied and documented (Buwalda et al., 2014). Hydrogels can be categorised into natural and synthetic (Zhu & Marchant, 2011). Alginates (Lee & Mooney, 2012), gelatin (Cheng, Lin, Ling, & Young, 2017), hyaluronic acid (I. L. Kim, Mauck, & Burdick, 2011) and chitosan (B. Ding et al., 2016) are the naturally occurring polymers and are completely biodegradable with good cell adhesion property (B. Ding et al., 2016). Naturals polymers effectiveness comes with a cost of immunogenicity and batch differences (Dimatteo, Darling, & Segura, 2018). On the other hand, the synthetic polymers like polyacrylamide, polymethyl methacrylate and polyvinyl alcohol show better mechanical properties and less immunogenicity issues. However, biochemical processing is required before in vivo application (Gyles, Castro, Silva, & Ribeiro-Costa, 2017). Thus, hybrid gels exhibit a mixed advantage of natural and synthetic polymers and boast great prospect in tissue repair and wound healing (Braun, Menges, Opoku, & Smith, 2013; Drury & Mooney, 2003; Sun et al., 2012).

Hydrocolloids are long chain hydrophilic polymers instantly dissolve or disperse into the solvent and swell significantly in water (Williams & Phillips, 2009). Hydroxyl group present in hydrocolloids absorb a lot of water thus, these are of great interest in food industry as the binding affinity towards water changes the whole texture and feel of the food product. Hydrocolloids are divided into fully-partial polysaccharides and categorised according to their origin such as tree exudate (gum Arabic, gum tragacanth, gum karaya), seaweed (alginate, agar-agar, carrageen) plant origin (pectin) and animal origin (gelatine and chitin) (Saha & Bhattacharya, 2010).

2.2.1 Alginate based

Bifidobacterium breve was protected against the low pH condition offered by simulated gastric juice alginate multilayer hydrogel beads were used. Using emulsion method, the probiotic strain was

encapsulated into spherical, smooth calcium alginate beads. Both the encapsulate and free culture were exposed to various pH conditions which simulated gastric and intestinal transit. Results showed that viability of the encapsulated *B. breve* were increased substantially than the free culture ones (Y. Li et al., 2017). Acidic condition inside the stomach is so extreme that single layer alginate beads render ineffective in maintaining the viability of the probiotic cells. Thus, Mokarram, Mortazavi, Najafi, and Shahidi (2009) in their work showed that increasing the encapsulation layer in alginate beads enhances the viability of probiotic microbes. Additionally, the degradation of single layer is faster compared to multilayer encapsulation, which was proved by Y. Li et al. (2012) under intestinal and colonic conditions of pH 6.8 and 7.2 respectively.

Dairy products are mainly used to deliver probiotics into human diet, but to consider population with lactose intolerance, fruit juices are an alternative source. However, this alternative source comes with a short coming of highly acidic pH which is a harmful environment for probiotic microbes (Perricone, Bevilacqua, Altieri, Sinigaglia, & Corbo, 2015). Alginate hydrogels have already proven the suitability to improve the viability of probiotics under pH stress conditions, thus has been investigated in fruit juices. *Lactobacillus plantarum* was encapsulated in single, double, and uncoated alginate beads, coating was done using chitosan. An ideal probiotic delivery system should be able to improve the survival rate of the probiotic organism under gastric environments as well as thermal treatment processes like pasteurisation which is often used in the manufacturing of fruit beverages (Petruzzi et al., 2017).

Bifidobacterium lactis, *B. longum*, *L. rhamnosus*, *L. salivarius*, *L. acidophilus*, *L. paracasei* are the various probiotic microbes which have been encapsulated into calcium alginate beads and exposed to higher temperatures like 65°C. The heat tolerance of encapsulated cells was investigated and it was evident that after 30 min of incubation at 65°C the encapsulated microbes showed improved rate of viability compared to the free cells. These results prove that calcium alginate beads possess good heat and acid tolerance which makes them one of the best carriers for probiotic delivery system. These crystals are well tolerated in all enteric simulated fluids such as gastric acid, bile and colonic secretions (W. K. Ding & Shah, 2007).

2.2.2 Xanthan-Based Hydrogels

Microencapsulation of *L. plantarum* was investigated with xanthan and alginate hybrid hydrogel. To research the acid tolerance of xanthan-alginate beads the coated probiotic cells as well as free cells were exposed to simulated gastric and colonic fluids. It was evident from the results that the viability of free cells was less than that of the encapsulated cells. Additionally, it was found that coating the Xanthan-alginate with chitosan improved the survivability of the encapsulated cells in the acidic environment. In case of the beads which were not coated with chitosan disintegrated in an hour and on the contrary the coated ones stayed until maximum release. Target specific delivery was achieved

by both type of beads. Furthermore, the encapsulated coated and uncoated beads were made to undergo thermal abuse at 75°C and 90°C for 30 sec and 5 sec correspondingly. It was concluded that the beads coated with polysaccharide (chitosan) had better thermal barrier capacity and improve the survival rate of L. plantarum (Fareez, Lim, Mishra, & Ramasamy, 2015).

Chitosan being cationic, can form physical hydrogels with anionic polysaccharide xanthan. Pediococcus acidilactici, a probiotic strain of bacteria was encapsulated into such physical hydrogel and was exposed to gastric simulated conditions. After the study the results showed that the encapsulated *P. acidilactici* showed higher viability than free cells in the gastric conditions. Additionally, the probiotic cells were completely released in intestinal conditions and negligible in gastric environment. This kind of positive and desired outcomes is correlated to the pH sensitive swelling mechanism of physical hydrogel formed between xanthan and chitosan. Moreover, the encapsulation efficiency after freeze-drying was also studied and a desired behaviour was obtained, giving good viability of probiotic cells even after drastic sub-freezing temperature (Argin, Kofinas, & Lo, 2014). These hydrogels have been used to encapsulate different probiotic strains such as *L. acidophilus* (H. Chen et al., 2015).

L. Chen, Yang, Song, Shu, and Chen (2017) studied the possibility of using the xanthan-chitosan hydrogels to enhance the viability of probiotic bacteria not only under stress conditions such as gastric environment, but also during storage representing conditions such as refrigeration and room temperature. In the study two types of beads were prepared, one was single layer (xanthan-chitosan) and other one was double layer (xanthan-chitosan-xanthan). Probiotic cells (*B. bifidum*) were encapsulated into both the types of beads and were stored in yogurt for 3 weeks at 4 and 25°C. The viability of *B. bifidum* encapsulated in both type of beads stored at refrigeration temperature i.e. 4°C has increased substantially in comparison to the free cells. Surprisingly, encapsulated cells survived better at 25°C (room temperature) than the free cells, where the level was way below the viable level indorsed by World Health organisation. Viability level wise both single- and double-layer encapsulation performed very well, but while examining the probiotic cell release profile the single-layer encapsulation had better release profile.

2.2.3. Pectin-Based Hydrogels

Pectin is another polysaccharide which can be used in combination with other non-polysaccharide polymers to encapsulate the probiotic microbes to act as a carrier for delivery. Gebara et al. (2013) formulated pectin hydrogel microparticles by using calcium chloride and a portion of microparticles were coated with whey protein. *L. acidophilus* was encapsulated into the coated and uncoated pectin-hydrogel microparticles and exposed to gastrointestinal conditions. The survivability of encapsulated *L. acidophilus* was improved in comparison to free probiotic cells. Remarkably, the pectin-hydrogel microparticles coated with whey protein did not provide any supplementary protection as intended to

the encapsulated microbes. Although the release profile of both the coated and uncoated was desirable and has reached maximum in intestinal fluid.

In another research R. Li et al. (2016) encapsulated *L. rhamnosus* into pectin hydrogel beads along with glucose. The encapsulated probiotics were subjected to simulated gastric and colonic environment containing all the digestive enzymes. As expected, the encapsulation provided better protection from the digestive enzymes and acids for the probiotic cells. Furthermore, addition of glucose into the beads even more improved the protection. The encapsulated beads were freeze dried store over a month to check the viability alterations whilst storage. Results showed that the survivability of the encapsulated probiotic cells which were freeze dried improved in comparison to free culture. So, it can be predicted that the addition of glucose can improve the viability and the encapsulated cells can have more stability if stored at ambient temperature.

Polysaccharide hydrogels as a potential probiotic delivery system have been well documented by many researchers. The studies have proven that the probiotic microbes encapsulated with these hydrogels can improve the viability of the cells during the gastrointestinal transit, as well as whilst storage and process temperature changes. Numerous polysaccharides are being tested and the list is increasing constantly. It can be concluded from the cited studies, the mixture of one or more polysaccharide is giving better protection against the tested condition rather than single polysaccharide hydrogel. Furthermore, addition of an extra component into the carrier system, particularly as a coating material may decrease the pore size thereby reducing the contact of the probiotic cells with the environmental stress outside.

Chapter 3

Materials and methods

3.1 Gel preparation

Gel was prepared using Disodium guanylate (DG) (Sigma-Aldrich) solution in RO water at 0.5M and 0.25M (duplicate) concentrations (20 ml each). Similarly, citric acid (Sigma-Aldrich) solution in RO water was prepared at 1M (duplicate) and 0.5M concentration (20 ml each). To initiate gelling, DG solutions at 0.5 and 0.25M (duplicate) concentration were mixed with citric acid solutions at 1M (duplicate) and 0.5M concentrations respectively. DG and citric acid were dissolved completely using vertexing (15 seconds) and heating in a water bath (at 50^{o}C until mixture become transparent) and placed at room temperature (22^{o}C) for 1 hr. A total of 40 ml of each combination was prepared to perform texture analyses and viscosity measurement of the gel.

Table 1: Novel gel code and composition

NG Code	Concentration of DG	Concentration of CA
NG I	0.25 M	0.5 M
NG II	0.25 M	1 M
NG III	0.5 M	1 M

3.2 Flow characteristics

The rheological properties of different gels were measured to characterize flow behaviour using a concentric cylinder viscometer which has an inner co-axial cylinder rotating in a stationary cylinder. The RM 100 Lamy rheometer was equipped with DN 33 spindle. Shear rate 12.9 s-1, 25.8 s-1, 51.6s-1, 77.5s-1 and 129s-1 for 30s were used to study the flow characteristics of the novel gel. Similarly, viscosity analysis of marketed Benzac cream were performed at similar shear rates for 30s.

3.3 Texture analysis

Gel mixtures were stored at 4°C for 24 hours before being tested regarding textural properties. The texture analysis of NG was carried out by using TA-XT2i Texture Analyser (Stable Micro Systems Ltd, Surrey, UK). The system was equipped with 5 kg loading cell and the gels were exposed to penetration at a continual crosshead speed of 2 mm/s to a distance of 10 mm with help of cylindrical plunger (p/5). Once the system attains the trigger force of 10g the cylindrical plunger starts to penetrate and rupture the gel sample until the specified distance of 10 mm. Data was extracted and scrutinised using exponent software to find out the hardness, firmness, rupture strength and cohesiveness.

3.4 Statsical analysis

Measurements were done in triplicates and one-way analysis of variance (ANOVA) was carried out using Minitab 18 (developed by Minitab LLC, Pennsylvania). Tukey comparison method was used to disclose significant differences ($p < 0.05$) among the samples.

Chapter 4

Results and discussion

4.1 Flow characteristics

Figure is illustrating the viscometrical behaviour of the novel gel at varying concentration of DG and CA. Gel containing DG (0.5 M) with CA (1 M) was found to be having highest apparent viscosity (2281 mPa.s at 12.9 s-1) at all shear rate. This defines a strong molecular structure in comparison to other concentration of DG and CA. When the shear rate was increased (12.9 to 129 s-1) a drop in viscosity was observed. However, the trend shown by the apparent viscosity was higher than other gel concentration. Gel containing DG (0.25 M) and CA (1 M) was also showing similar shear thinning behaviour as the applied shear stress increased. Though, the apparent viscosity was found to be less than the gel with higher concentration of DG (0.5 M). As expected, gel with lower concentration of DG (0.25 M) and CA (0.5 M) the apparent viscosity in comparison to other two gels dropped even further retaining the shear thinking behaviour. Surprisingly, none of the combination showed a Newtonian behaviour of no change in the apparent viscosity with varying shear stress. It can be inferred that viscosity highly depend on the shear rate and on the concentration of DG and CA. So, when the concentration if principal ingredients like DG and CA varies it strongly affects the rheological behaviour of the novel gel.

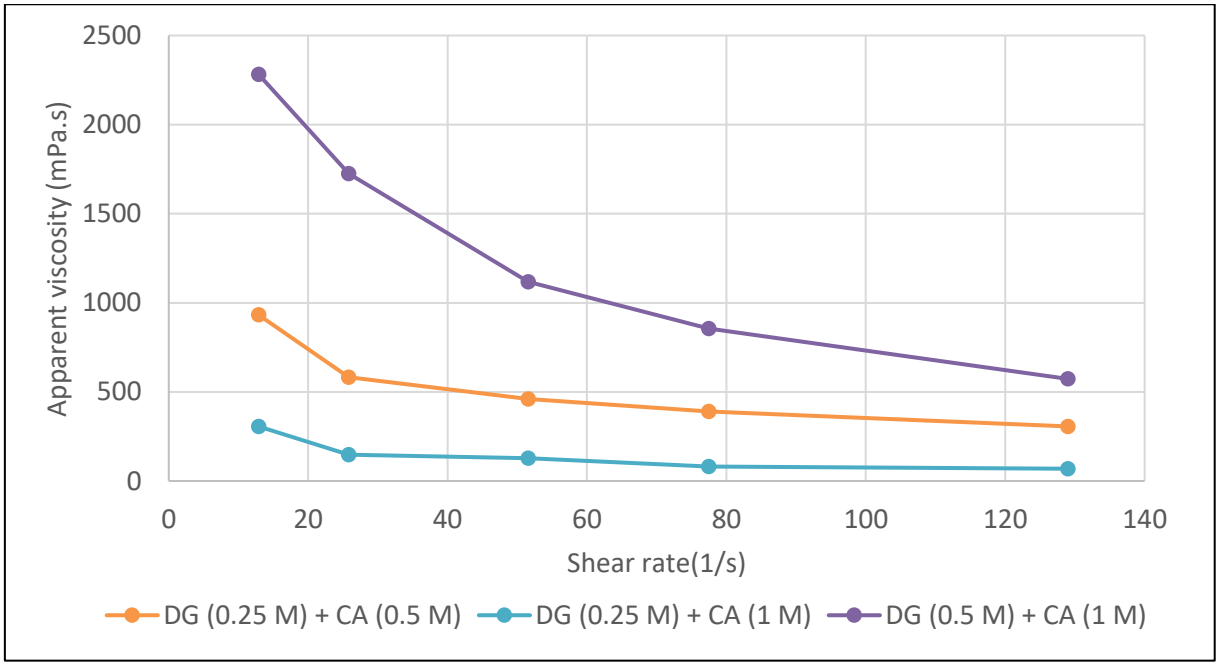

Figure 2: Viscosity of gels containing DG and citric acid at different concentrations

When gel was prepared by keeping DG concentration same (0.25 M) and varying concentration of CA (0.5 M and 1 M), the gel was showing shear thinning behaviour with respect to increasing shear stress.

Novel gel prepared with CA concentration at 1 M showed highest viscosity at all shear rates (932 mPa.s at 12.9 s-1 and 306 mPa.s at 129 s-1). According to the results its evident that change in CA concentration induced drastic changes in viscosity of novel gel retaining the shear thinning behaviour. So, it can be understood from the results that the CA concentration is very imperative because when the CA concentration was reduced to 0.5 M there was an overall drop in viscosity across the shear stress applied. Correspondingly, when the CA concentration was kept constant (1 M) and DG concentration was varied (0.25 M and 0.5 M), the gel prepared with higher concentration of DG had higher viscosity at all shear rates. Also, it can be observed from the magnitude of increase in the viscosity that modulating the concentration of DG and CA is very imperative to control the rheological behaviour of the gel.

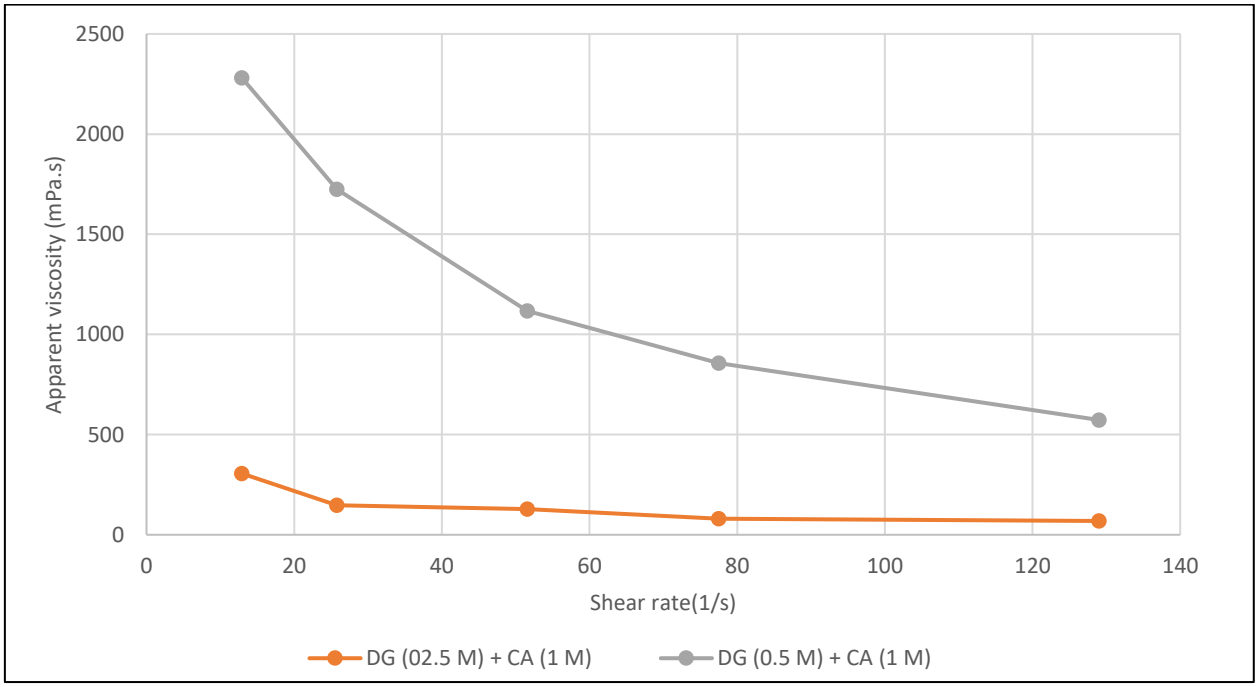

Figure 3: Viscosity of gels containing fixed CA concentration with different DG concentration

Table 2: Apparent viscosity of all NG samples

Shear rate	DG (0.25 M) + CA (1 M)	DG (0.25 M) + CA (0.5 M)	DG (0.5 M) + CA (1 M)
12.9	306	932	2281
25.8	148	583	1724
51.6	128	461	1117
77.5	81	390	856
129	69	306	573

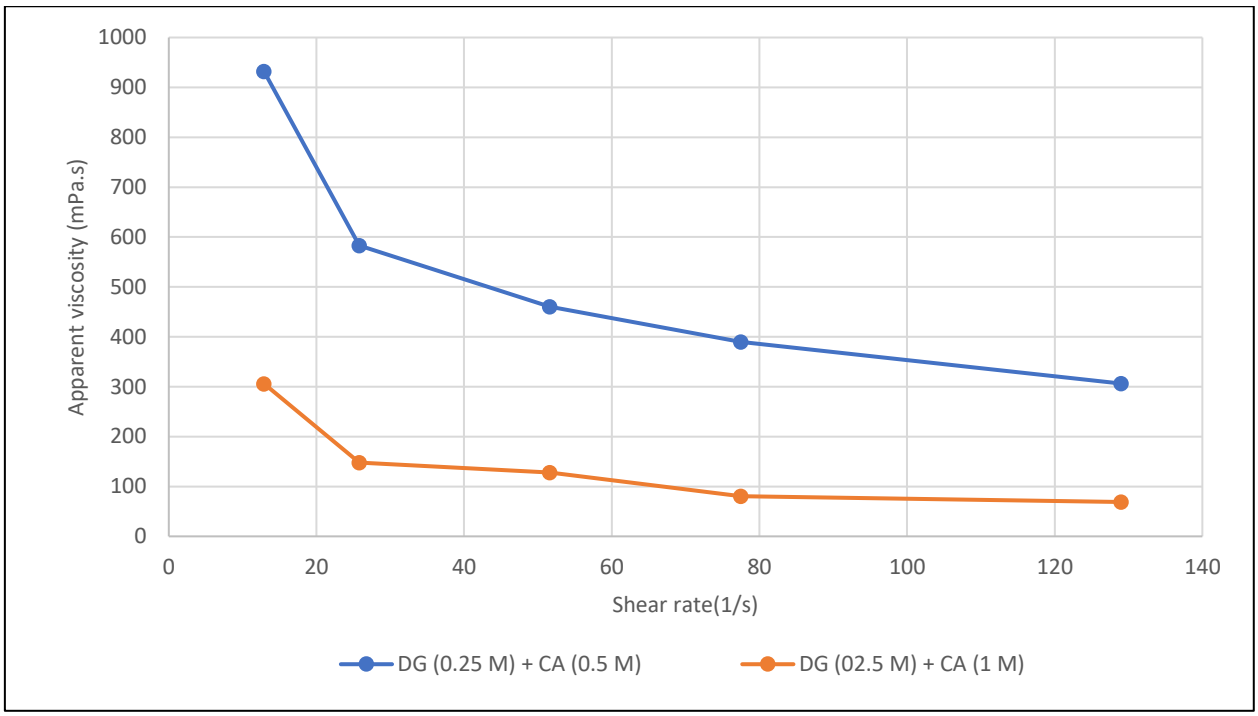

Figure 4: Viscosity of gels containing fixed DG concentration with different CA concentration

Temperature dependent change in viscosity was also studied by using novel gel prepared from DG (0.25 M) and CA (0.5 M). Gel was prepared in two parts; one part was refrigerated at 4℃ and the other was kept at room temperature prior to the test. The result was showing substantial difference in the viscosity when the temperature of storage was varied. The viscosity of gel stored at 4℃ was high at lower shear rates in comparison to the one stored at room temperature. However, once the shear rate was higher the viscosity of both the gels were comparatively same.

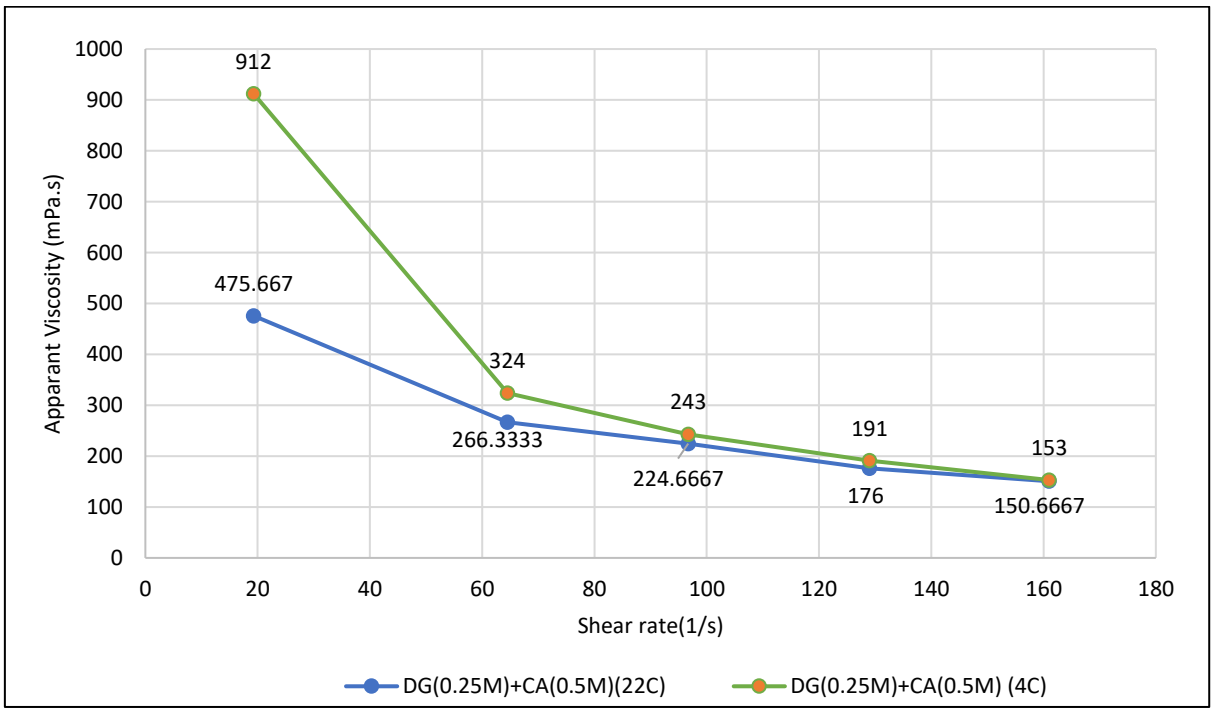

Figure 5: Effect of different temperature on the gel viscosity

Gel formation is the process which involves the association of the polymer chains and results in the formation of a 3-dimensional network responsible for immobilization of water within the system (Lewis, 1996) . According to the viscosity analysis conducted to observe the flow behaviour of newly developed gel containing DG and citric acid revealed that gel showed shear thinning behaviour which indicates that viscosity decreases as shear rate increase. As compared to the other gels (Disodium guanylate with malic acid, lactic acid, gallic acid, ascorbic acid), new gel containing disodium guanylate and citric acid achieved high apparent viscosity which indicate the formation of stronger 3-dimensional network as compared to others. Moreover, we investigated the effect of disodium guanylate and citric acid concentration on the viscosity and as we decreased the concentration of disodium guanylate (0.25 M to 0.06M) and citric acid (0.5M to 0.125M) simultaneously, viscosity reduced due the weak bonding within the gel system. Furthermore, we monitored the impact of disodium guanylate concentration on viscosity on gel by reducing the concentration of citric acid alone (0.5M to 0.125M) and according to the results, formed gel still showed shear thinning behaviour with decreasing viscosity as shear rate increases. However, reducing the citric acid concentration reduces the apparent viscosity.

4.2 Texture analysis

Under texture analysis mainly two parameters are analysed and investigated; rupture strength and brittleness. Rupture strength means the amount of force required to rupture the unbroken gel. In simple words it defines the hardness of the gel (Aarstad et al., 2017). Hydrogels and hydrocolloids applied to the bruised or wounded biological surface should possess a microgel structure which could resist the stress produced by the skin movement, also at the same instance stick to the skin for a longer duration (Islam, Rodríguez-Hornedo, Ciotti, & Ackermann, 2004). While developing an ideal topical formulation, when the goal is an extended retention time at the wounded site for the success of treatment. To achieve this, there should be a stable balance between the adhesiveness and cohesiveness. At this point textural analysis gives useful data about these mechanical properties to execute the development properly. Hardness or adhesiveness of the gel defines its ability to stick on to the site of application and is directly related to the polymer concentration (Jones, Woolfson, & Brown, 1997a, 1997b). The mechanical properties like viscosity and texture tend to depend on the composition of hydrogels. During the evaluation physical evaluation of properties of gels, texture analysis gives a wide array options to modify the measurement to attain reproducible and validated results which are very desirable. As literature suggests, texture of a gel can be assessed in its original form or compressing it into beads or tablets (Coviello et al., 2005; Jones, Woolfson, & Djokic, 1996).
Highest hardness was observed with the gel prepared with higher concentration of CA (1 M) and lower concentration of DG (0.25 M). However, the viscosity was found more in case of the gel prepared by similar CA concentration but 0.5 M DG. This result was in accordance with the published data presented by Chelikani et al. (2021) where they used exactly same concentration of the gel. In normal

gelling agents such as gelatine the internal structure aligns in the direction of the force which in turn results in reduced hardness (Ahmed, 2017; Renard, van de Velde, & Visschers, 2006). FTIR and NMR data produced by Chelikani et al. (2021) emphasize the hydrogen bonds formed by NG is not in the direction of the force or flow thus attaining higher hardness and unique gelation characteristics (Roy, Kar, Das, & Datta, 2020). Temperature can possibly affect the mechanical properties like hardness, firmness and cohesiveness of the gel (Dingley, Stephenson, Allender, Dawson, & Williams, 2018). During rheological study when the viscosity was measured at approximately 4℃, the resulting apparent viscosity was twice as that of gel kept at room temperature. This change in viscosity can be co related to the hydrogen bond formation. As the temperature decreases the hydrogen bond formation is increased due to reduction in the energy between the molecules leading to inter connected cross linkages which at last increases the viscosity (Yan, Schröter, Herbst, Binder, & Thurn-Albrecht, 2014). This kind of phenomenon is seen in freezing of water (Bai & Yonker, 1998; Ohtaki, 2003).

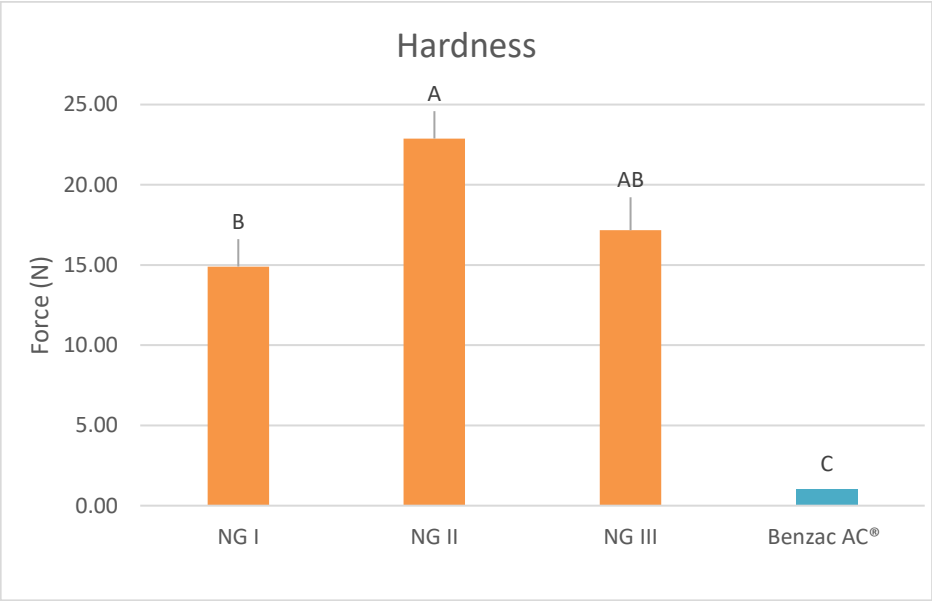

Figure 6 : Texture (with respect to force) comparison of NG at different concentration

Table 3: Tukey comparison of hardness of different NG concentration with Benzac AC®

Sample	N	Mean	Grouping	
NG I	2	22.88	A	
NG II	2	17.17	A	B
NG III	2	14.904		B
Benzac AC®	2	1.00		C

*Means that do not share a letter are significantly different

After the texture analysis of the NG, irrespective of concentration, when the schematic graph was studied, the firmness of the gel was high but the cohesiveness was very less which in turn suggests the

non-sticky nature of the gel (Figure 6). Also, from the rheological data it was evident that the gel was showing a shear thinning nature and was taking a lot of time to rebuild the viscosity (thixotropic) after being sheared. This kind of structure recovery is seen in gel formulations with stabilisers such as microcrystalline cellulose or sodium carboxymethylcellulose (Adeyeye, Jain, Ghorab, & Reilly, 2002). So, the possibility of usage as a strong protective film can be explored as the gels with thixotropic or pseudoplastic behaviour show resistance to spreadability (Gaspar & Maia Campos, 2003). The textural property of the NG was compared with a marketed anti acne gel Benzac AC® (Figure 7), which showed less hardness compared to NG but good cohesiveness. This might be due to the acrylate polymer used in the formula. So, if NG can be formulated with some biodegradable polymer as an adhesive then a desirable topical delivery system can be formulated.

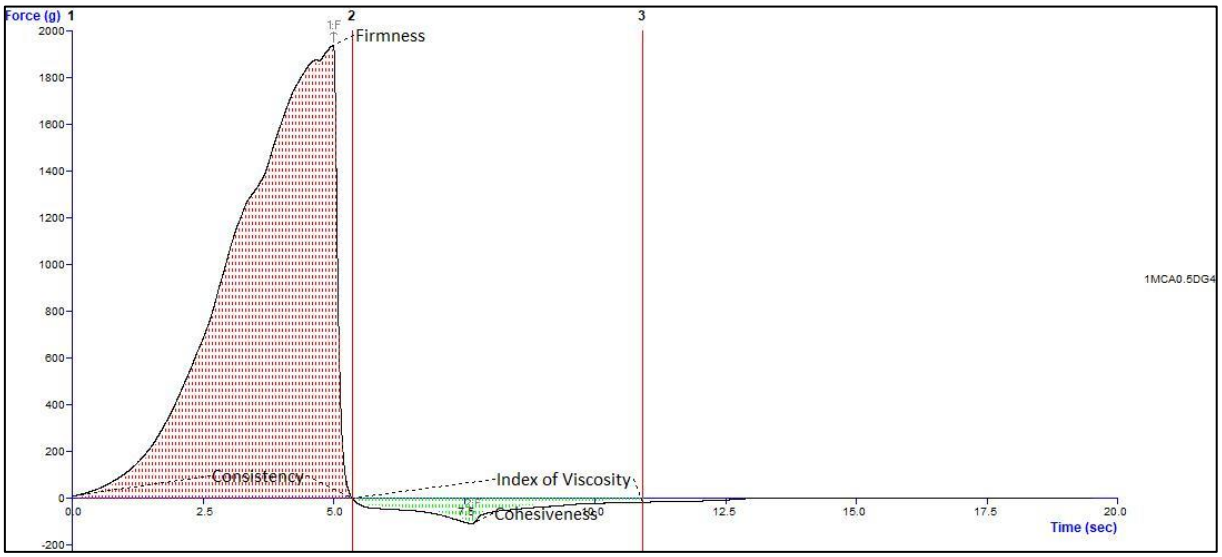

Figure 7 : Texture analysis schematic of NG III

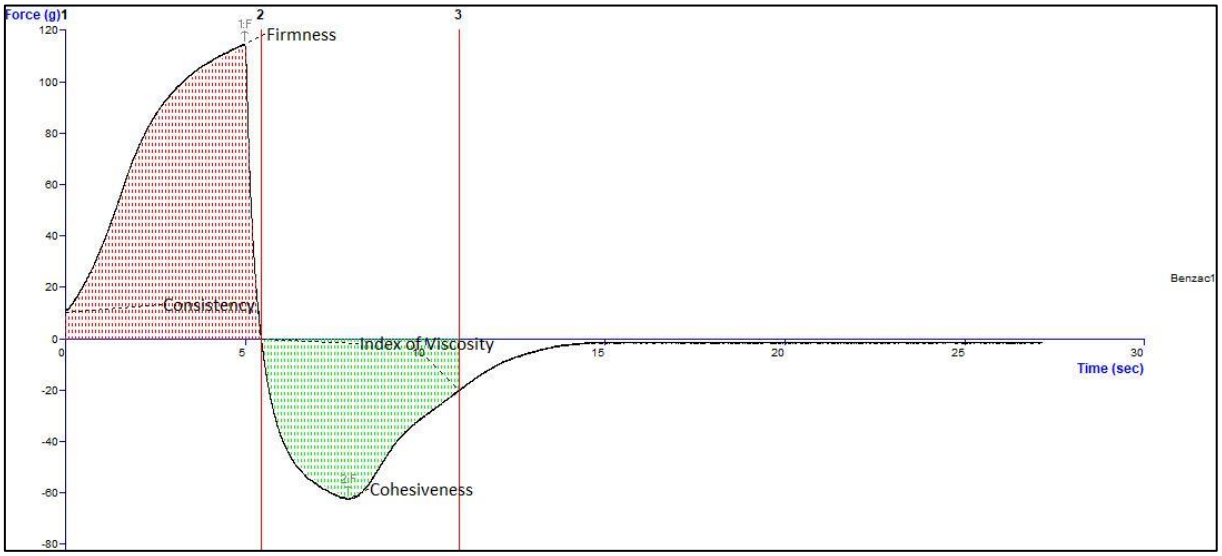

Figure 8: Texture analysis schematic of Benzac AC®

Chapter 5

Conclusion

Physical properties like texture and viscosity of novel gel at different concentration was studied. When comparing the results with the study carried out by Chelikani et al. (2021) the textural and rheological properties are much superior to the gelatine, a well-established stabiliser-plasticiser available in the market. Also, considering the ingredients used to prepare the gel, this can be used as an alternative to animal-derived gels. As, the molecular structure formed by combining DG with CA, there is a possibility of combining DG with other food acids like ascorbic or lactic acids which have molecular weight rendering stronger gels. The information gathered from the above research can be used characterise and optimise hydrogel for topical probiotic delivery to treat lesions and acnes. Texture and rheology give direct information about the adhesive and cohesive behaviour of the gel and directly define the outcome of intended therapy. These physical properties not only affect the physical nature of the intended formulation, but also the release of the incorporated probiotic microbes from the delivery system. So, as to achieve this next step would be to optimise the formulation from a pharmaceutical point of view covering the drug release and bio adhesion. Additionally, in vivo release testing also should be carried out to evaluate the efficacy of the formulated product to prove the theory.

Chapter 6

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Appendix

A.1 Raw statistical data for hardness

One-way ANOVA: Hardness versus Sample

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
Sample	3	DG (0.25 M) + CA (0.5 M), DG (0.25 M) + CA (1 M), DG (0.5 M) + CA (1 M)

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	2	67.493	33.747	11.35	0.040
Error	3	8.917	2.972		
Total	5	76.410			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.72406	88.33%	80.55%	53.32%

Means

Sample	N	Mean	StDev	95% CI
DG (0.25 M) + CA (0.5 M)	2	14.904	0.841	(11.025, 18.784)
DG (0.25 M) + CA (1 M)	2	22.88	2.26	(19.00, 26.76)
DG (0.5 M) + CA (1 M)	2	17.17	1.76	(13.29, 21.05)

Pooled StDev = 1.72406

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping	
DG (0.25 M) + CA (1 M)	2	22.88	A	
DG (0.5 M) + CA (1 M)	2	17.17	A	B
DG (0.25 M) + CA (0.5 M)	2	14.904		B

Means that do not share a letter are significantly different.

A.2 Texture analysis raw data

Table A. 1 Texture analysis raw data from exponent software

Test ID	Batch	Firmness	Consistency	Cohesiveness	Index of Viscosity
		g	g.sec	g	g.sec
		Force 1	Area F-T 1:2	Force 2	Area F-T 2:3
Start	1MCA0.5DG				
1MCA0.5DG1	1MCA0.5DG	1460.16	3563.12	-68.21	-271.56
1MCA0.5DG2	1MCA0.5DG	1581.54	3948.68	-80.31	-365.55
End	1MCA0.5DG				
Average:	1MCA0.5DG	1520.85	3376.3	-77.57	-269.03
Start	1MCA0.25DG				
1MCA0.25DG1	1MCA0.25DG	2497.66	5816.41	-18.86	-46.01
1MCA0.25DG2	1MCA0.25DG	2170.9	5118.9	-16.96	-11.92
End	1MCA0.25DG				
Average:	1MCA0.25DG	2572.78	5467.65	-17.91	-28.97
Start	0.5MCA0.25DG				
0.5MCA0.25DG1	0.5MCA0.25DG	1625.33	5296.28	-44.15	-67.85
0.5MCA0.25DG2	0.5MCA0.25DG	1878.69	5960.31	-36.94	-50.05
End	0.5MCA0.25DG				
Average:	0.5MCA0.25DG	1752.01	5628.3	40.545	58.95
Start	Benzac				
Benzac1	Benzac	115.12	398.03	-62.62	-237.69
Benzac2	Benzac	88.53	331.15	-51.67	-228.63
End	Benzac				
Average:	Benzac	101.82	364.59	-57.14	-233.16
End of Test Data					

A.3 Texture analysis raw data – graphs

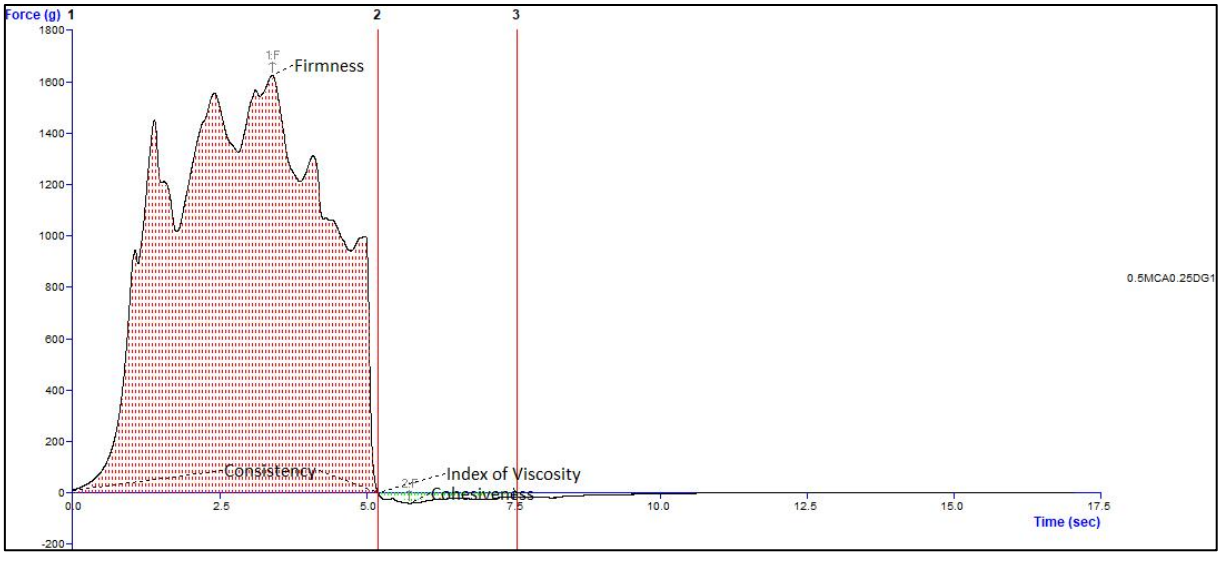

Figure A. 1 Texture analysis schematic of NG I (Trial 1)

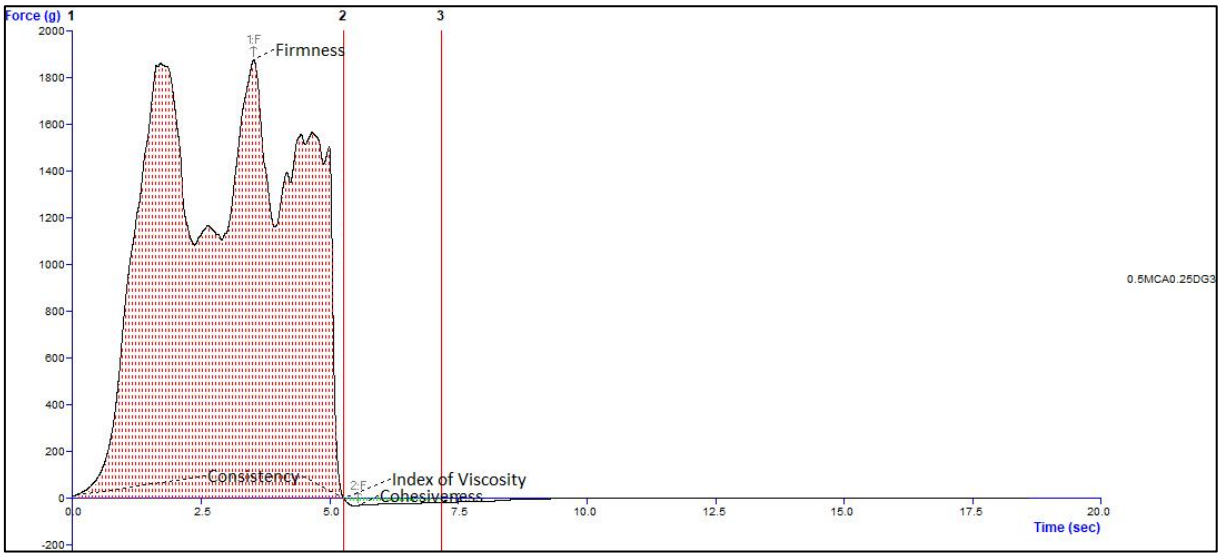

Figure A. 2 Texture analysis schematic of NG I (Trial 2)

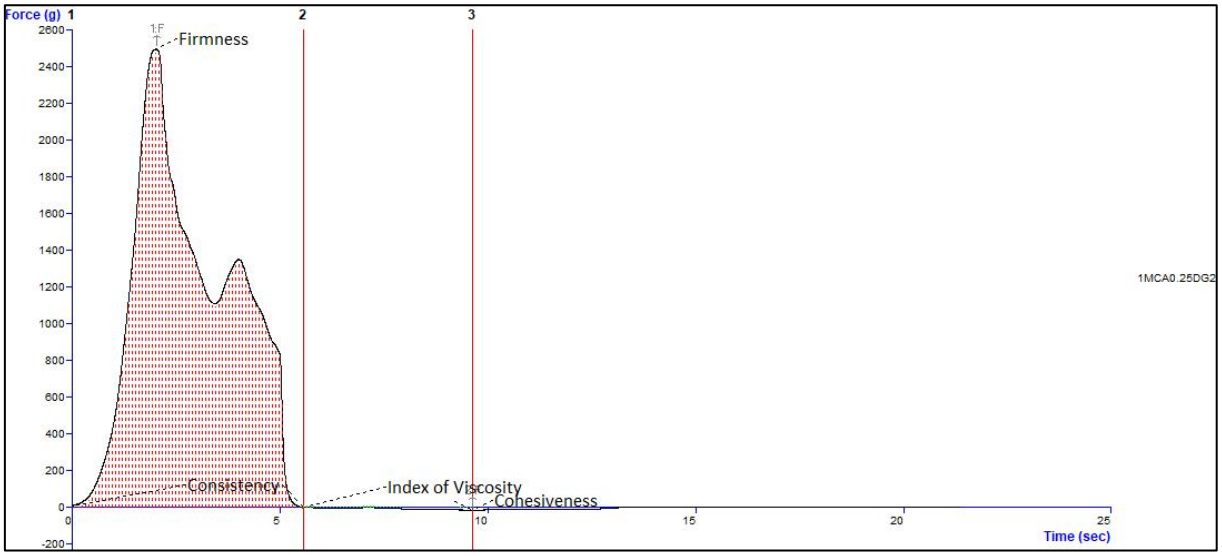

Figure A. 3 Texture analysis schematic of NG II (Trial 1)

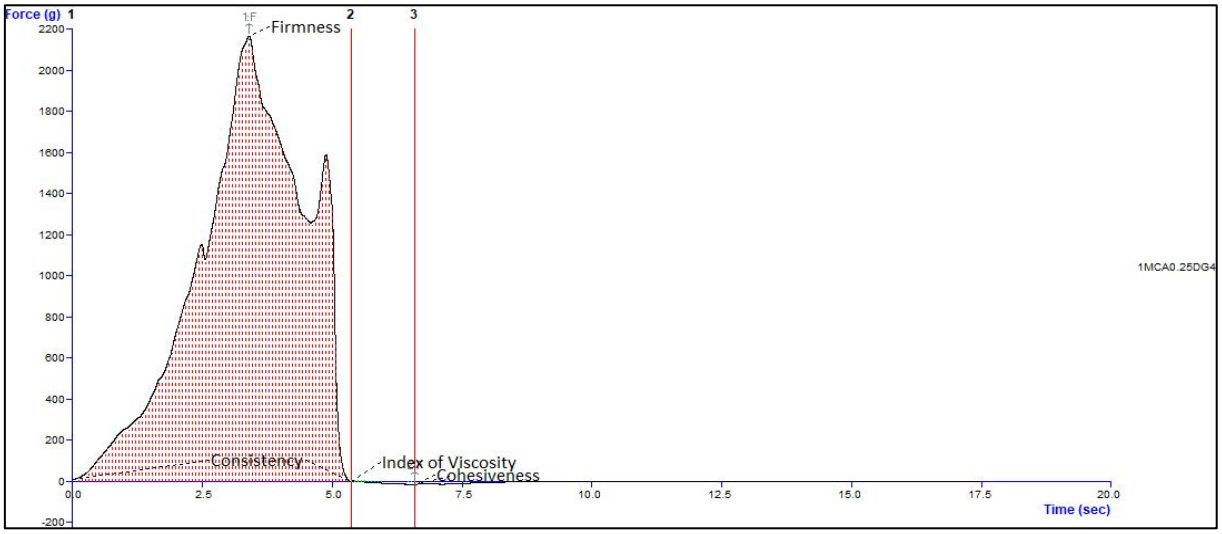

Figure A. 4 Texture analysis schematic of NG II (Trial 2)

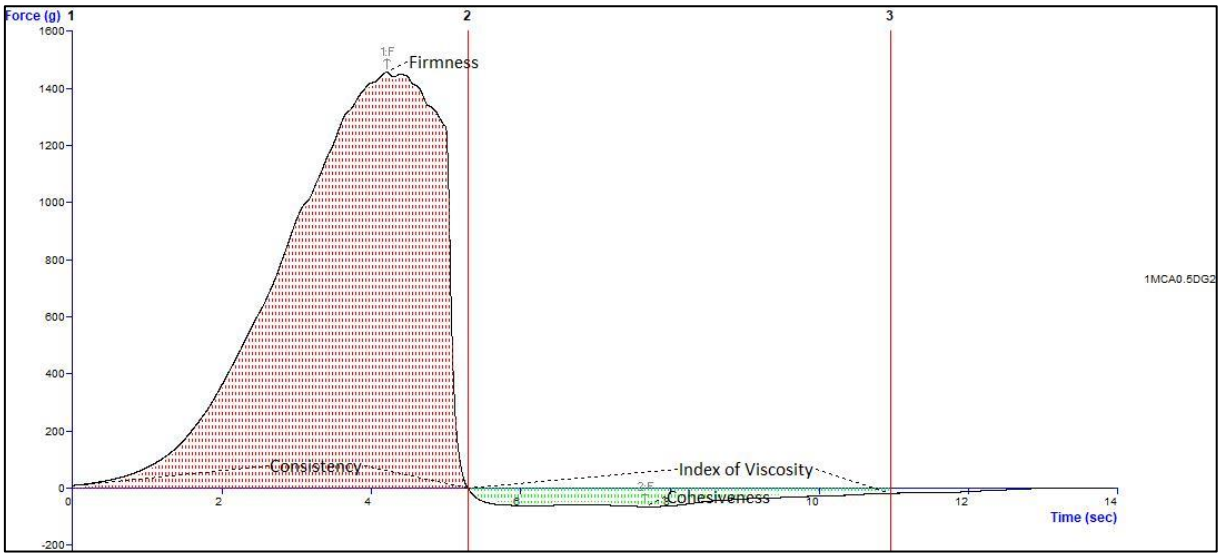

Figure A. 5 Texture analysis schematic of NG III (Trial 1)

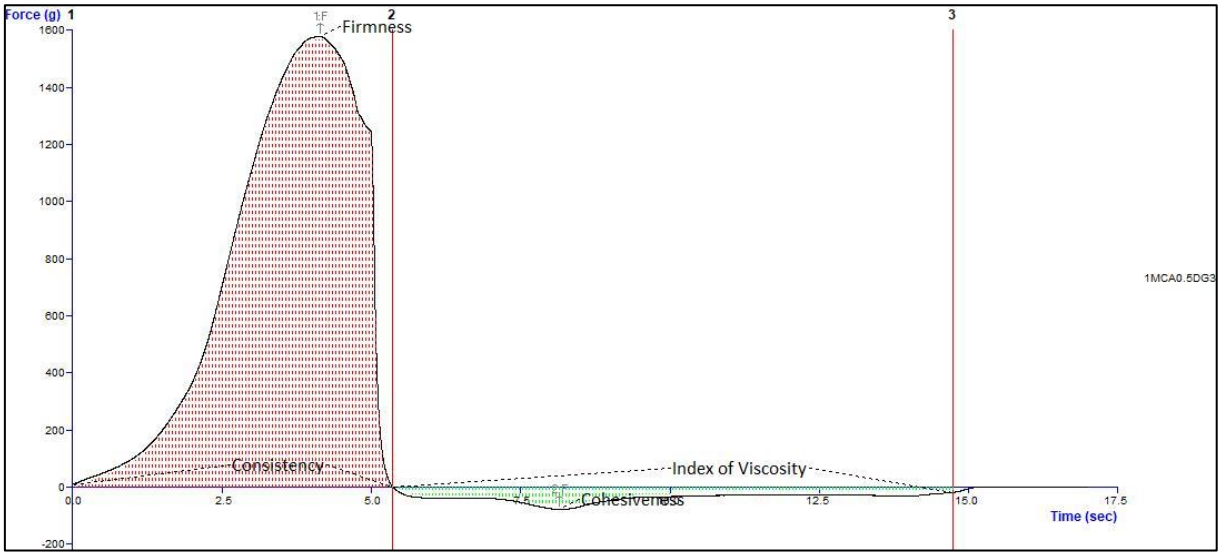

Figure A. 6 Texture analysis schematic of NG III (Trial 2)

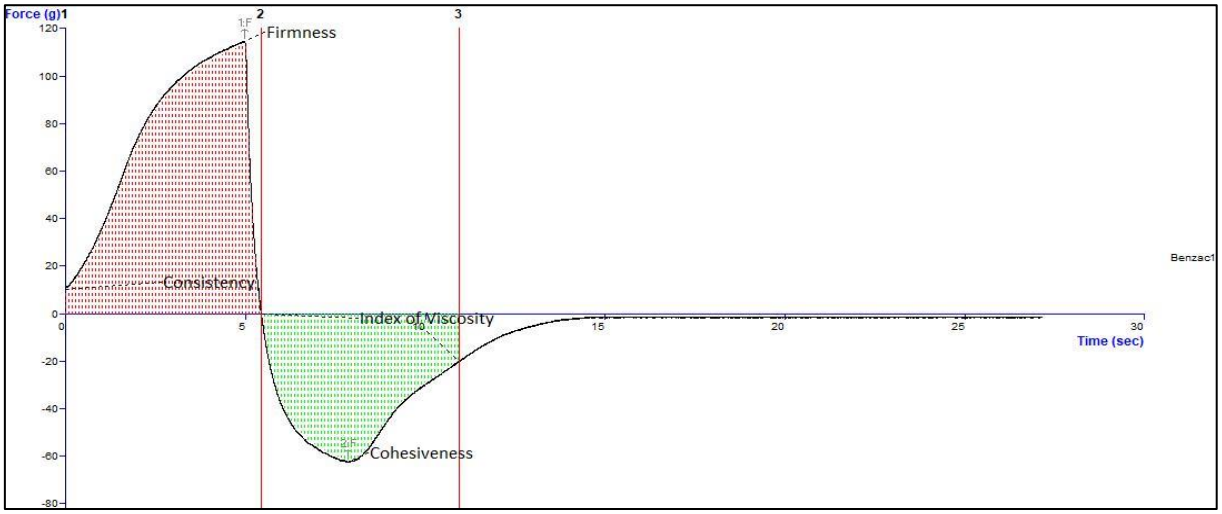

Figure A. 7 Texture analysis schematic of Benzac AC® (Trial 1)

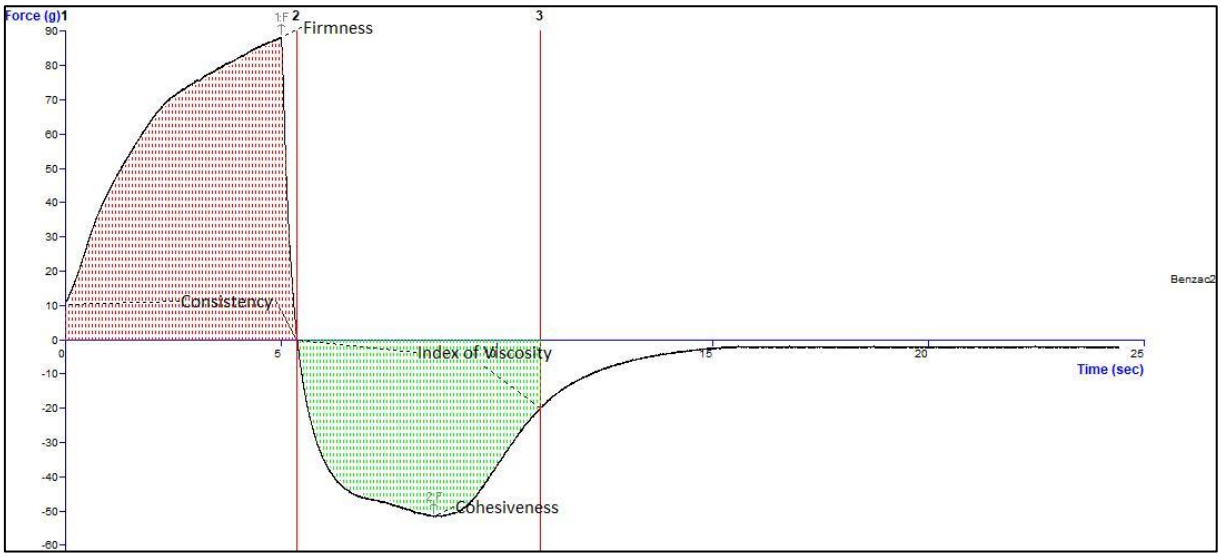

Figure A. 8 Texture analysis schematic of Benzac AC® (Trial 2)